Copper toxicity and sulfur metabolism in Chinese cabbage

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Chapter 6.

Atmospheric H_2S nutrition hardly affects the toxicity and impact of copper on sulfur metabolism in Chinese cabbage

Abstract

H_2S exposure did not affect plant biomass production, dry matter, pigment and total sulfur content, if plants were grown at an ample sulfate supply to the root. There was a direct interaction between atmospheric H_2S and pedospheric sulfate utilization and H_2S exposure resulted in a downregulation of the sulfate uptake capacity and the expression of Sultr1;2 in the root. H_2S exposure did not affect the toxicity of Cu^{2+}, indicating that a higher sulfur status of the plant did not have any significance in Cu toxicity. There was a substantial increase in thiol content of the shoot (2-fold) and to a lesser extent of that in the root in H_2S exposed plants. If plants were simultaneously exposed to H_2S and enhanced Cu^{2+} concentrations, the thiol accumulation in the root even further increased. Both in absence and presence of H_2S, high Cu^{2+} concentrations (> 10 μM) resulted in an upregulation of the sulfate uptake capacity, however, a H_2S-induced partial downregulation of the sulfate uptake capacity also occurred at all Cu^{2+} concentrations. The H_2S-induced downregulation of the expression of Sultr1;2 was hardly affected at increasing Cu^{2+} concentrations. Sulfate deprivation of Chinese cabbage resulted in a decreased biomass production, a decreased shoot to root ratio, an increased dry matter content (especially in the shoot), and in a decreased pigment and sulfur metabolite content, and in a strongly increased sulfate uptake capacity of the root. The Group 1, 2 and 4 sulfate transporters were all upregulated in both root and shoot. The impact of sulfate deprivation on growth and the expression and activity of the sulfate transporters were hardly further affected at enhanced Cu^{2+} levels. If sulfate-deprived plants were simultaneously exposed to H_2S, biomass production was quite similar to that of sulfate-sufficient plants, though the shoot to root ratio remained lower. The upregulation of the expression of the sulfate transporters upon sulfate deprivation and that of APS reductase in absence and presence of enhanced Cu^{2+} in the shoot was largely or completely alleviated upon H_2S exposure. At all conditions, the regular signal transduction pathway of sulfate transporters and APS reductase was overruled at high Cu tissue levels.

Introduction

Enhanced Cu^{2+} concentrations (≥ 5 μM) in the root environment were toxic for Chinese cabbage, which could in part be ascribed to a Cu-induced disturbed development of the chloroplasts, resulting in chlorosis and a reduced biomass production (Sheldon and Menzies 2005; Yruela 2005; Chapter 3 and 4). Upon UV radiation, enhanced Cu^{2+} concentrations had negative synergistic effects in Chinese cabbage, which was probably largely due to a higher accumulation of Cu in both root and shoot (Chapter 4). Sulfate deprivation had a more rapid negative effect on plant biomass production than an enhanced Cu^{2+} level in Chinese cabbage (Chapter 5). There was a strong accumulation of water-soluble non-protein thiols in the root and, to a lesser extent in the shoot upon Cu^{2+} exposure. The increase in thiol content in Chinese cabbage could only partially be ascribed to a Cu-induced synthesis of phytochelatins (Chapter 3). Phytochelatins and other thiols may be involved in the binding of excessive free Cu and its detoxification, and the Cu-induced synthesis of these thiols might require an enhanced sulfate uptake and assimilation (Inouhe 2005; Lee and Kang 2005; Sirko and Gotor 2007). Indeed, enhanced Cu contents in the root affected the regulation of the uptake and distribution of sulfate; there was an increase in expression and activity of the high affinity sulfate transporter Sultr 1;2. This increase was accompanied with an increase in total sulfur content in the shoot, which was mainly due to an accumulation of sulfate (Chapter 3 and 4). However, it needs still to be resolved to what extent the upregulation of the sulfate transporters was the consequence of a higher sulfur demand at higher Cu concentrations or the result of an interference/reaction of Cu with the signal compounds regulating the expression and activity of the sulfate transporters (Chapter 3).

In addition to sulfate taken up by the root, plants are able to utilize the foliarly-absorbed sulfurous air pollutants (SO_2, H_2S) as sulfur source for growth (De Kok *et al*. 2002, 2007, 2009; Yang *et al*. 2006; Koralewska *et al*. 2008). Atmospheric H_2S is directly metabolized with high affinity into cysteine and subsequently into other organic sulfur compounds (De Kok *et al*. 1998, 2002, 2007, 2009). There was a direct interaction between atmospheric and pedospheric sulfur utilization in *Brassica*, and H_2S exposure resulted in downregulation of sulfate uptake by the root and its and assimilation in the shoot

(Westerman *et al*. 2000, 2001; Buchner *et al*. 2004; De Kok *et al*. 2007; Koralewska *et al*. 2008). In absence of sulfate in the root environment, an atmospheric level of 0.2 µl l^{-1} H_2S appeared to be sufficient to cover the sulfur demand for growth of *Brassica* (Koralewska *et al*. 2008).

In the present chapter, the interaction between pedospheric sulfate and atmospheric H_2S nutrition, and enhanced Cu^{2+} levels was studied, in order to get insight into the significance of the plant sulfur status in Cu toxicity, and the interference of Cu with the signal transduction pathway regulating the expression and activity of the sulfate transporters (and APS reductase).

Plant growth conditions

10-day-old seedlings of Chinese cabbage were transferred to a 25% Hoagland solution at 0.5 mM sulfate (+S, sulfate-sufficient) or 0 mM sulfate (-S, sulfate-deprived), containing supplemental concentrations of 0, 5, 10 and 15 µM $CuCl_2$ in 13 l containers (10 sets of plants per container, 3 plants per set) in fumigation cabinets and fumigated with 0 or 0.2 µl l^{-1} H_2S for 11 days.

Results

Impact of Cu, H_2S and sulfate deprivation on growth, pigment content and Cu content

Exposure of Chinese cabbage to 0.2 µl l^{-1} H_2S for 11 days did not affect plant biomass production, shoot to root ratio, dry matter content and pigment content at sulfate-sufficient condition (Fig. 1 and 2). However, the Cu content was slightly higher in root and slightly lower in shoot at sulfate-sufficient conditions upon H_2S exposure (Fig. 4). If plants were exposed to enhanced Cu^{2+} concentrations (≥ 5 µM) at sulfate-sufficient conditions, the biomass production of both root and shoot was decreased in both absence (+S) and presence of H_2S (+S, H_2S), whereas the shoot to root ratio was hardly affected (Fig. 1). At ≥ 10 µM Cu^{2+} there was an increase in dry matter content in both root and shoot (Fig. 2).

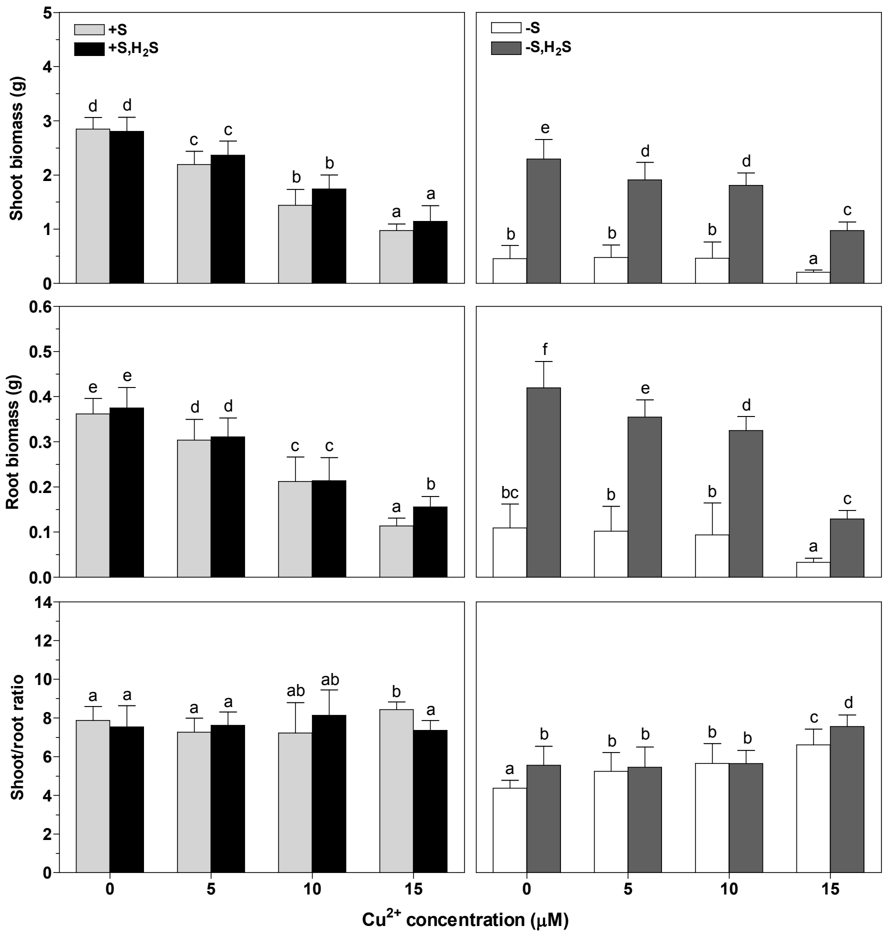

Fig. 1. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on biomass production of Chinese cabbage. 10-day-old seedlings of Chinese cabbage were grown on a 25% Hoagland solution containing 500 μM sulfate (+S, light grey bars), fumigated with 0.2 μl l^{-1} H_2S (+S,H_2S, black bars) or 0 μM sulfate (-S, white bars), fumigated with 0.2 μl l^{-1} H_2S (-S,H_2S, dark grey bars) for 11 days. Plants were exposed to 0 to 15 μM Cu^{2+} in the root environment. The initial shoot and root weight was 0.059 ± 0.004 and 0.009 ± 0.003 g fresh weight, respectively. Data on biomass production (g FW) and shoot/root ratio represent the mean of 2 experiments with 9 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

At sulfate-sufficient conditions pigment content was only decreased at 15 μM Cu^{2+}, whereas the chlorophyll *a*/*b* and chlorophyll/carotenoid ratio were hardly affected in both absence and presence of H_2S (Fig. 3). The Cu content in both root and shoot of Chinese cabbage increased with the Cu^{2+} concentration (Fig. 4). At sulfate-sufficient conditions, the Cu content was increased up to 40-fold in root and up to 5-fold in shoot at 15 μM Cu^{2+}. H_2S exposure of Cu^{2+}-exposed plants resulted in a lower Cu content at 15 μM and in root, and in shoot at all Cu^{2+} concentrations (Fig. 4).

The plant biomass production was strongly decreased upon a 11-day sulfate deprivation, whereas the shoot to root ratio was decreased and the dry matter content substantially increased (Fig. 1 and 2). Exposure of sulfate-deprived plants to enhanced Cu^{2+} concentrations resulted in a decreased biomass production of both root and shoot at 15 μM, whereas the shoot to root ratio was increased at ≥ 5 μM, but dry matter content of both root and shoot was hardly affected (Fig. 1 and 2). Sulfate deprivation resulted in a decreased pigment content, and upon exposure to Cu^{2+} the chlorophyll content and the chlorophyll *a/b* and chlorophyll/carotenoid ratio were hardly further affected (Fig. 3). The Cu content of sulfate-deprived plants was rather high in both root and shoot; it increased up to 56-fold in root and up to 5.5-fold in shoot at 15 μM Cu^{2+} (Fig. 4). H_2S exposure of sulfate-deprived (-S, H_2S) plants alleviated the development of sulfur deficiency symptoms. The plant biomass production, dry matter and pigment content were quite similar to that of sulfate-sufficient (+S) plants, however, the chlorophyll *a/b* was significantly higher in the presence of H_2S (Fig. 1, 2 and 3). A simultaneous exposure of sulfate-deprived plants to Cu^{2+} and H_2S resulted in a decreased plant biomass production at ≥ 5 μM, an increased shoot to root ratio at 15 μM, and an increased dry matter content of root at ≥ 10 μM (Fig. 1 and 2). There was a decrease in pigment content at ≥ 10 μM Cu^{2+}, however, the chlorophyll *a/b* and chlorophyll/carotenoid ratio were hardly affected (Fig. 3). The Cu content of H_2S-fumigated, sulfate-deprived plants was higher in root, and at enhanced Cu^{2+} concentrations, its content increased in both root and shoot. However, the Cu content of H_2S-fumigated, sulfate-deprived plants remained significantly lower than that of sulfate-sufficient plants at all Cu^{2+} concentrations (Fig. 4). The excessive Cu taken up by the root was relatively immobile and only a minor proportion was transferred to the shoot (Fig. 4). The latter further decreased with increasing Cu^{2+} concentrations in the root environment at both sulfate-sufficient and sulfate-deprived conditions (Fig. 4).

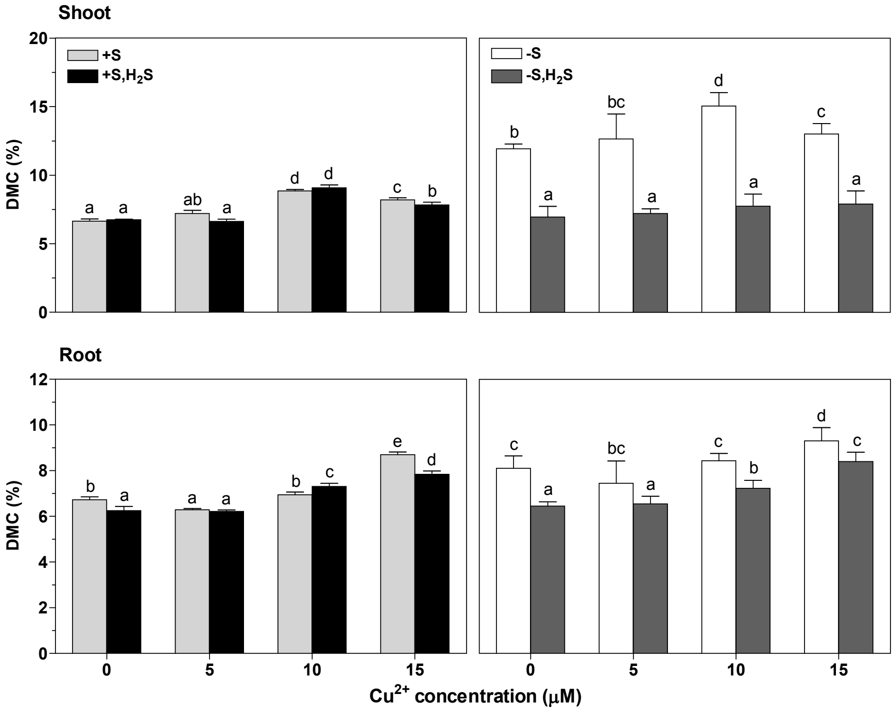

Fig. 2. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on dry matter content of Chinese cabbage. For experimental details see legends Fig. 1. Data on dry matter content (DMC; %) represent the mean of 2 experiments with 9 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

Impact of Cu, H_2S and sulfate deprivation on nitrogen and sulfur metabolite content

The content of nitrate, amino acids and total sulfur was not affected in sulfate-sufficient, H_2S-exposed plants (Fig. 5 and 6). However, H_2S exposure resulted in a slightly lower sulfate content of both root and shoot, a strong increase in the thiol content of the shoot, and to a lesser extent in the root (Fig. 6 and 7). Exposure to Cu^{2+} at enhanced concentrations (≥ 10 μM) resulted in a decreased nitrate content of both root and shoot in absence (+S) and presence of H_2S (+S, H_2S). The amino acid content was hardly affected upon Cu^{2+} exposure in both root and shoot in absence and presence of H_2S (Fig. 5). The total sulfur content of the shoot, both in absence and presence of H_2S, was substantially increased at ≥ 10 μM Cu^{2+}, whereas that of the root remained unaltered (Fig. 6). The increase in total sulfur content upon exposure to Cu^{2+} could for the greater part be attributed to an enhance sulfate content (Fig. 6). There was an increase in water-soluble non-protein thiol content in the root and to a lesser extent in the shoot at ≥ 5 μM Cu^{2+} at sulfate-sufficient conditions. The increase in thiol content of the root was further increased upon a simultaneous exposure to Cu^{2+} and H_2S, the thiol content of shoot was hardly further affected (Fig. 7).

Sulfate-deprived plants (-S) contained very low levels of total sulfur, sulfate and thiols in both root and shoot. Moreover, there was a strong increase in amino acid content, whereas the nitrate content was hardly affected (Fig. 5 and 6). If sulfate-deprived plants were exposed to enhanced Cu^{2+} concentrations, the total sulfur and thiol content in shoot and sulfate content in both root and shoot were hardly affected (Fig. 5, 6 and 7). However, the total sulfur and thiol content in the root was increased at ≥ 5 μM and the nitrate content decreased at 15 μM, and the nitrate content in the shoot increased at ≥ 5 μM Cu^{2+} (Fig. 5, 6 and 7). H_2S exposure of sulfate-deprived plants (-S, H_2S) resulted in a substantial increase in total sulfur and thiol content of both root and shoot, whereas the sulfate content remained low (Fig. 6). The amino acid content was quite similar to that of sulfate-sufficient plants (Fig. 5). A simultaneous exposure of sulfate-deprived plants to Cu^{2+} and H_2S resulted in a slight decrease in nitrate content in both root and shoot at ≥ 15 μM, whereas the amino acid, total sulfur and sulfate contents were hardly affected. Moreover, there was a strong increase in thiol content in the root at ≥ 5 μM, and a slight increase in the shoot at ≥ 10 μM upon a simultaneous Cu^{2+} and H_2S exposure of sulfate-deprived plants (Fig. 5, 6 and 7).

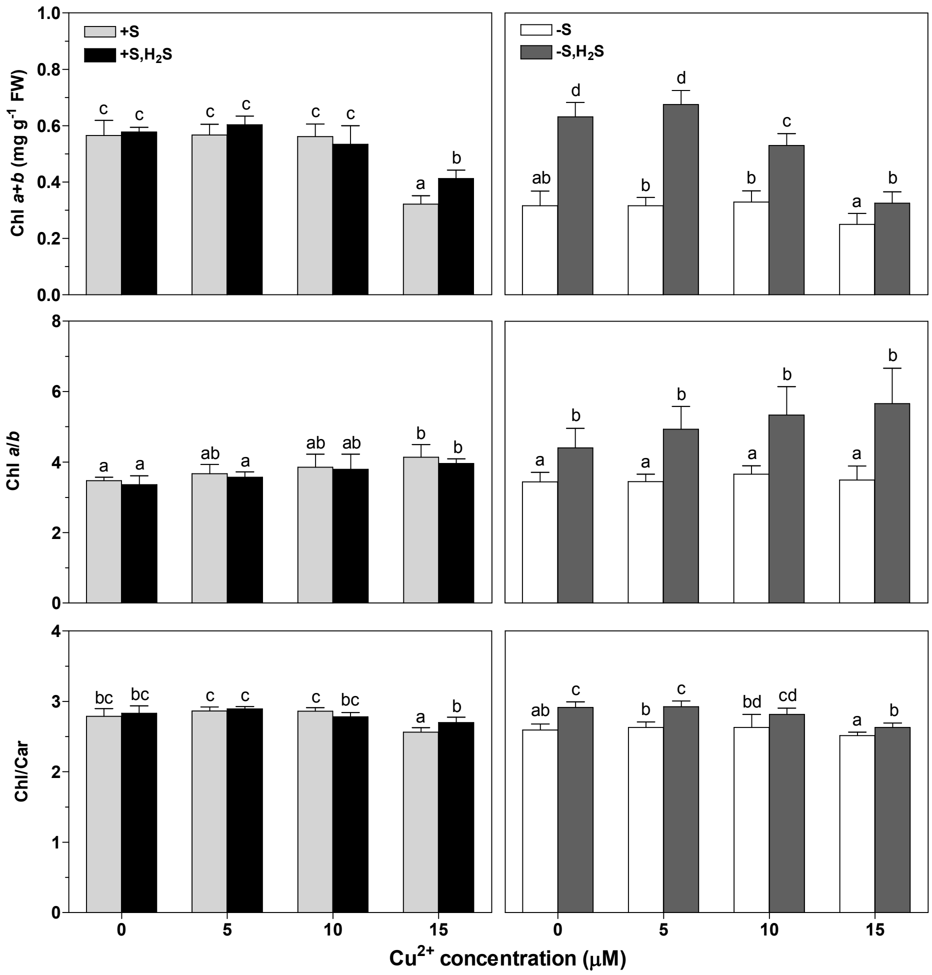

Fig. 3. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on pigment content of Chinese cabbage. For experimental details see legends Fig. 1. Data on chlorophyll content (chl *a+b*; mg g^{-1} FW), chlorophyll *a* to chlorophyll *b* ratio (Chl *a/b*), and chlorophyll to carotenoid ratio (Chl/Car) represent the mean of 2 experiments with 3 measurements in each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

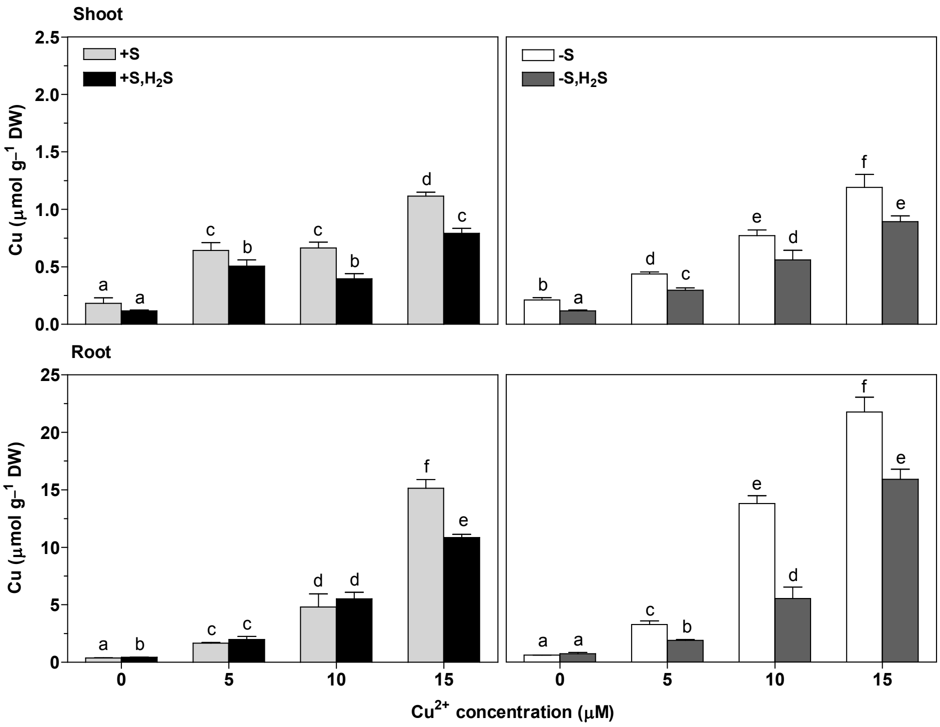

Fig. 4. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on copper content of Chinese cabbage. For experimental details see legends Fig. 1. Data represent the mean of 3 measurements with 9 plants each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

Impact of Cu, H_2S and sulfate deprivation on sulfate uptake capacity and expression of sulfate transporters and APS reductase

Exposure of Chinese cabbage to H_2S resulted in a downregulation of the sulfate uptake capacity at sulfate-sufficient conditions (+S; Fig. 8). Both in absence and presence of H_2S, concentrations > 10 μM Cu^{2+} resulted in an upregulation of the sulfate uptake capacity, however, the H_2S-induced partial downregulation of the sulfate uptake capacity also occurred at all Cu^{2+} concentrations. There was an increase in sulfate uptake capacity at enhanced Cu^{2+} concentrations in both absence (+S) and in presence of H_2S (+S, H_2S) at ≥ 10 μM

Cu^{2+} (Fig. 8). This increase in sulfate uptake capacity was accompanied with an enhanced expression of Sultr1;2 in root, whereas Sultr1;1 was hardly expressed at sulfate-sufficient conditions. The expression of Sultr1;2 was downregulated by 70% upon H_2S exposure and its expression was increased by 190% upon exposure to 15 µM Cu^{2+} in root (Fig. 9). H_2S exposure to sulfate-sufficient Chinese cabbage resulted in a downregulation of expression of the sulfate transporters and that of APS reductase in both root and shoot (Fig. 9). The expression of Sultr2;2 was enhanced by 120% at ≥ 5 µM Cu^{2+} in root, whereas Sultr2;2 was hardly expressed in the shoot of Chinese cabbage. The Cu-induced increased expression of Sultr2;2 was absent upon H_2S exposure (Fig. 9). The expression of Sultr4;1 increased with 50% in the root at 15 µM Cu^{2+} in absence and presence of H_2S, whereas, its expression in the shoot was slightly decreased (30%). The expression of Sultr4;2 was increased by 70% in root at 15 µM Cu^{2+} in absence and presence of H_2S, whereas, its expression was hardly expressed in the shoot (Fig. 9). The expression of APS reductase was increased by 60% in root at 15 µM Cu^{2+} in absence and presence of H_2S, whereas its expression was hardly affected by Cu^{2+} exposure and upon H_2S exposure down regulated by 70% in shoot (Fig. 9).

Sulfate deprivation (-S) resulted in an increased sulfate uptake capacity of the root of Chinese cabbage, which was even higher upon H_2S exposure (Fig. 8). Exposure of sulfate-deprived plants to enhanced Cu^{2+} concentrations did not affect the sulfate uptake capacity. A simultaneous Cu^{2+} and H_2S exposure of sulfate-deprived plants resulted in an increased sulfate uptake capacity only at 15 µM (Fig. 8). The expression of the sulfate transporters and APS reductase was strongly enhanced upon sulfate deprivation in both root and shoot (Fig. 9). The expression of Sultr1;1 was decreased by 80% at 15 µM Cu^{2+} in root and was hardly affected upon H_2S exposure at sulfate-deprived conditions. Sultr2;2 was slightly expressed at sulfate-deprived conditions but it was hardly affected by Cu^{2+} exposure in both root and shoot. Upon H_2S exposure Sultr1;2 and Sultr2;2 were hardly expressed in shoot in both absence and presence of enhanced Cu^{2+} concentrations in the root environment (Fig. 9).

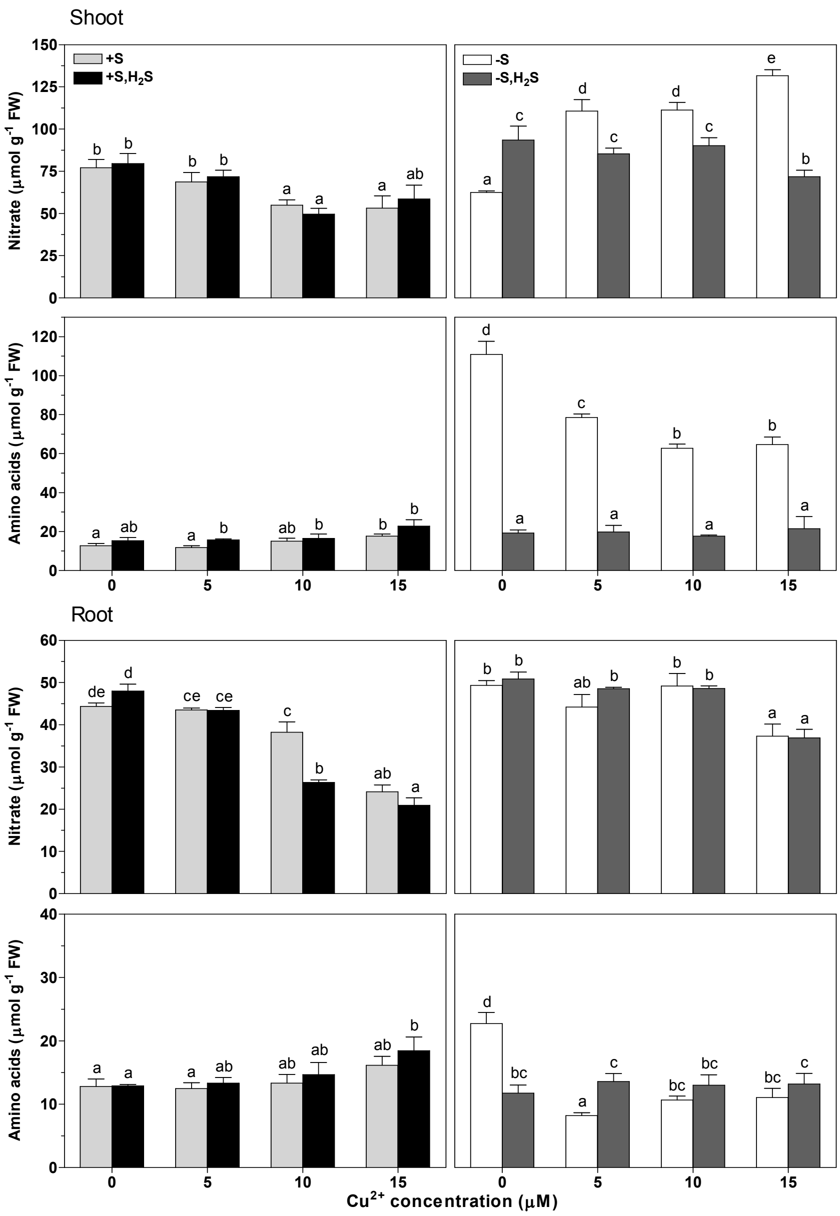

Fig. 5. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on nitrate and amino acids content of Chinese cabbage. For experimental details see legends Fig. 1. Data on nitrate and amino acids content (µmol g^{-1} FW) represent the mean of 3 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

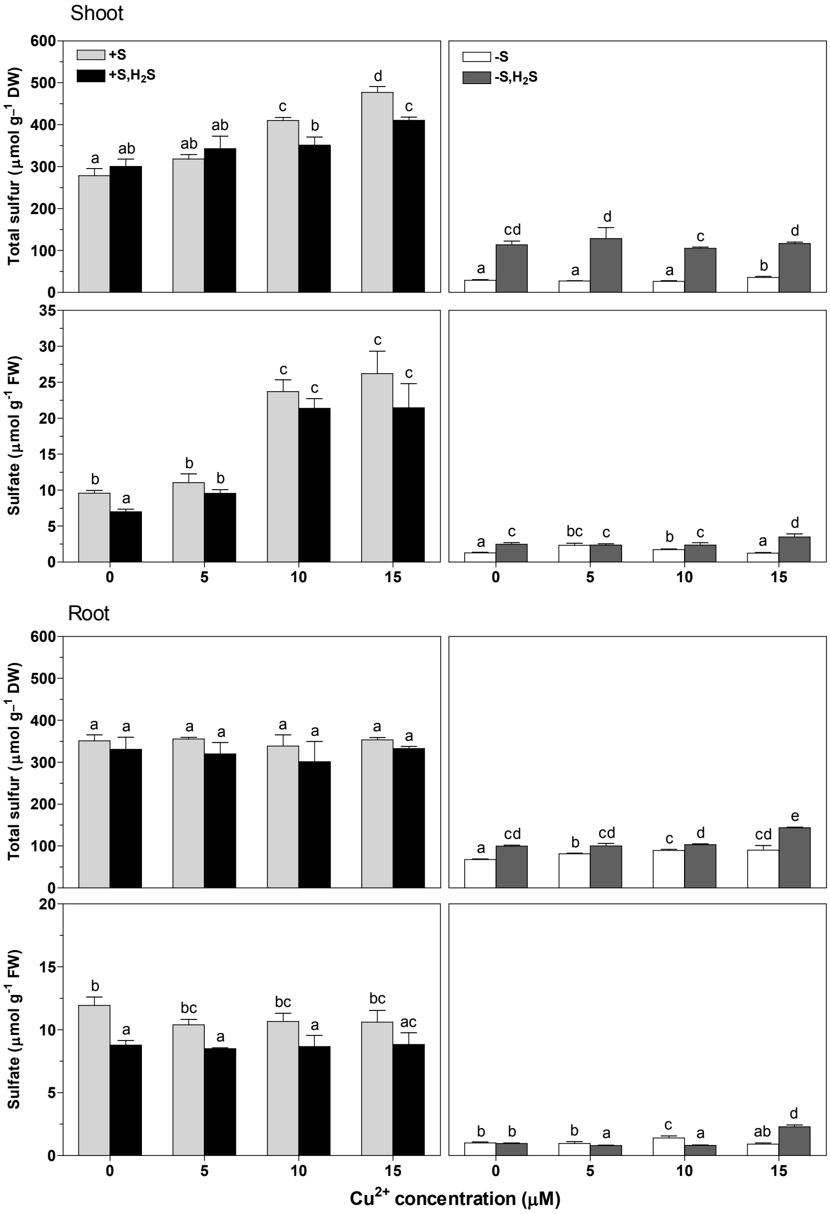

Fig. 6. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on total sulfur and sulfate content of Chinese cabbage. For experimental details see legends Fig. 1. Data on total sulfur (μmol g^{-1} DW), and sulfate content (μmol g^{-1} FW) represent the mean of 3 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

Upon sulfate deprivation, the expression of Sultr4;1 and Sultr4;2 in root was hardly affected by Cu^{2+} and by a simultaneous Cu^{2+} and H_2S exposure. The expression of these transporters was decreased by 80% upon H_2S exposure in shoot in absence and presence of enhanced Cu^{2+} concentrations. The expression of APS reductase was hardly affected in root upon Cu^{2+} and upon a simultaneous Cu^{2+} and H_2S exposure at sulfate-deprived conditions. However, its expression was downregulated by 75-90% in shoot upon H_2S exposure in absence and presence of enhanced Cu^{2+} concentrations in the root environment (Fig. 9).

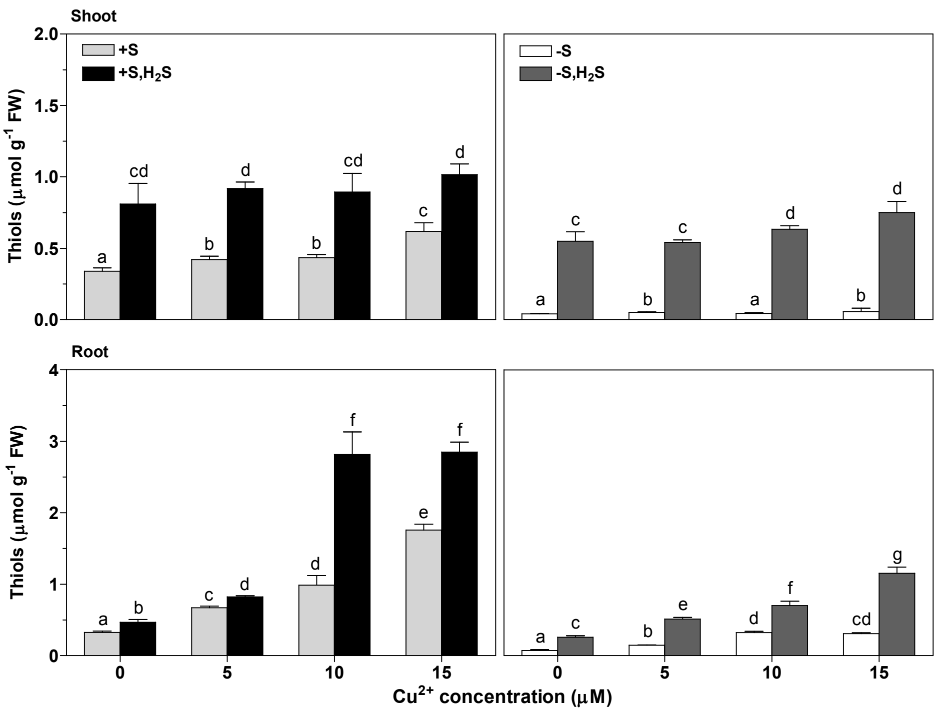

Fig. 7. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on thiol content of Chinese cabbage. For experimental details see legends Fig. 1. Data on thiol content (μmol g^{-1} FW) represent the mean of 3 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

Discussion

Similar to previous observations, Chinese cabbage was able to utilize foliarly absorbed H_2S and replace sulfate, taken up by the root, as sulfur source for growth (Koralewska *et al*. 2008). At sulfate-sufficient conditions, H_2S exposure did not affect plant biomass production, dry matter, pigment and total sulfur content, but it resulted in a substantial increase in thiol content of the shoot (2-fold) and to a lesser extent of that in the root. If sulfate-sufficient plants were simultaneously exposed to H_2S and enhanced Cu^{2+} concentrations in the root environment, the thiol accumulation in the root was further increased. Exposure of sulfate-sufficient Chinese cabbage to H_2S did not affect the toxicity of Cu^{2+} and the Cu-induced decrease in plant biomass production was quite comparable, even though the Cu content of the shoot was significantly lower upon H_2S exposure. This indicated that a higher sulfur status of the plant did not have any significance in Cu toxicity.

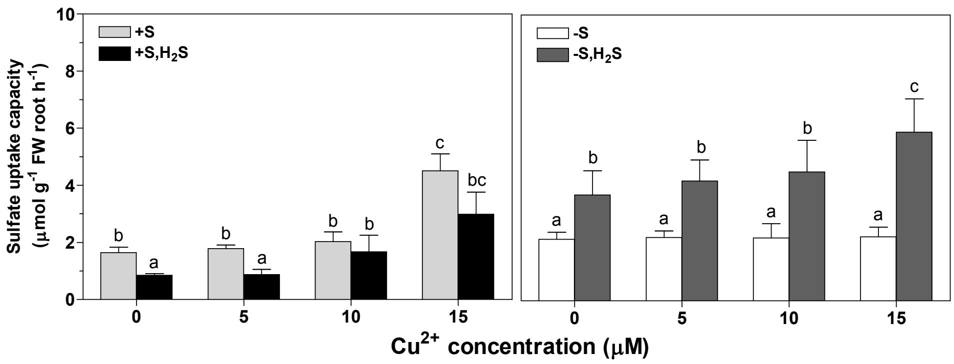

Fig. 8. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on sulfate uptake capacity of Chinese cabbage. For experimental details see legends Fig. 1. Data on sulfate uptake capacity (µmol g^{-1} FW root h^{-1}) represent the mean of two independent experiments with 3 measurements with 3 plants each (± SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

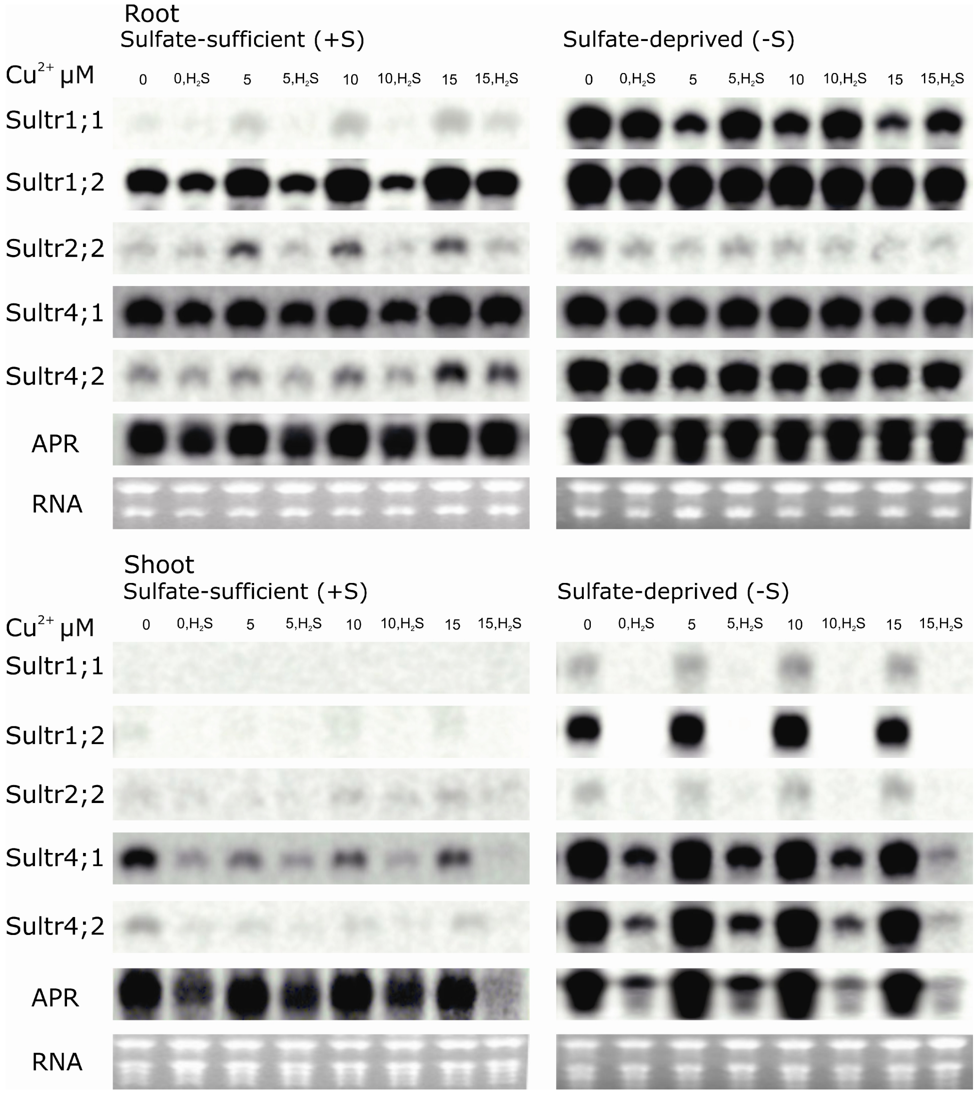

Fig. 9. Impact of Cu^{2+} and H_2S exposure and sulfate deprivation on mRNA abundance of sulfate transporters (Sultr) and APS reductase (APR) (Northern-blot analysis) in the root and shoot of Chinese cabbage. Equal RNA loading was determined by ethidium bromide staining of gels (shown in the bottom panels). A representative set of data from two independent experiments is given. For experimental details see legends Fig. 1.

Similar to previous observations, there was a direct interaction between atmospheric H_2S and pedospheric sulfate utilization in Chinese cabbage (Westerman *et al*. 2000, 2001; Buchner *et al*. 2004; De Kok *et al*. 2007; Koralewska *et al*. 2008). At sulfate-sufficient conditions, H_2S exposure resulted in a downregulation of the sulfate uptake capacity and the expression of Sultr1;2 in the root. Both in absence and presence of H_2S, increasing Cu^{2+} concentrations resulted in an upregulation of the sulfate uptake capacity. However, a H_2S-induced partial downregulation of the sulfate uptake capacity occurred at all Cu^{2+} concentrations. The H_2S-induced downregulation of the expression of Sultr1;2 was hardly affected at increasing Cu^{2+} concentrations and it even depressed the Cu-induced upregulation of this transporter. Moreover, the Cu-induced upregulation of Sultr1;1 and Sultr2;2 were alleviated upon H_2S exposure, the latter transporter is involved in the vascular transport of sulfate (Buchner *et al*. 2004; Parmar *et al*. 2007; Koralewska *et al.* 2010).

The expression of Sultr4;1 and Sultr4;2 was upregulated at 15 μM Cu^{2+} in root at sulfate-sufficient conditions. Generally, Group 4 sulfate transporters are only upregulated upon sulfur limitation, when plants remobilize and/or redistribute available sulfate, for instance from vacuoles (Buchner *et al*. 2004; Parmar *et al*. 2007; Koralewska *et al*. 2009a,b, 2010; Stuiver *et al*. 2009; Chapter 5).

Sulfate deprivation of Chinese cabbage resulted in a decreased biomass production, a decrease in shoot to root ratio, an increased dry matter content (especially in the shoot) and in a decrease in pigment and sulfur metabolite content. As observed before, sulfate deprivation resulted in a strongly increased sulfate uptake capacity of the root, and the Group 1, 2 and 4 sulfate transporters were all upregulated in both root and shoot (De Kok *et al*. 1997; Westerman *et al*. 2000; Buchner *et al*. 2004; Yang *et al*. 2006; Koralewska *et al*. 2007, 2008; Chapter 5). Similar to previous observations (Chapter 5), the consequences of sulfate deprivation on growth and the expression and activity of the sulfate transporters were hardly further affected by enhanced Cu^{2+} levels.

If sulfate-deprived plants were simultaneously exposed to H_2S, plant biomass production was quite similar to that of sulfate-sufficient plants. However, the shoot to root ratio remained lower, demonstrating that upon H_2S exposure in the absence of sulfate in the root environment, plants still invested relatively more biomass in root, even though the sulfur supply was sufficient to cover the sulfur demand for growth (Buchner *et al*. 2004; Koralewska *et al*. 2007, 2008). If plants were grown with H_2S as the sole sulfur source, the impact of enhanced Cu^{2+} concentrations on biomass production was comparable to that observed for sulfate-sufficient plants and the degree of Cu toxicity was similar. However, the upregulation of the expression of the sulfate transporters upon sulfate-deprivation and APS reductase in absence and presence of enhanced Cu^{2+} in the shoot was largely or completely alleviated upon H_2S exposure.

It is generally presumed that metabolic products of sulfate assimilation, *viz.* thiols as sulfide, cysteine, and glutathione act as signals in the regulation of the expression and activity of the sulfate transporters (Hawkesford and De Kok 2006). However, at enhanced Cu^{2+} concentrations, there was hardly any relation between the abundant thiol levels and the expression and activity of the sulfate transporters at sulfate-sufficient, sulfate-deprived conditions, both in presence and absence of H_2S. Apparently, the regular signal transduction pathway of the sulfate transporters was overruled at high Cu tissue levels. The expression and activity of APS reductase is generally downregulated at enhanced thiol levels (Westerman *et al*. 2001; Durenkamp *et al*. 2007; Koralewska *et al*. 2008). However, in sulfate-sufficient plants its expression was hardly affected in the root in presence of H_2S and enhanced Cu^{2+} concentrations, irrespective of the high thiol levels present under these conditions. Only in the shoot the increase in thiol content upon H_2S exposure was accompanied with a slight decrease in expression of APS reductase.

Despite the observations that enhanced Cu^{2+} concentrations interfered with the regulation of the uptake and assimilation of sulfur in plants, there was no direct relation between their sulfur status and Cu toxicity. Evidently, total sulfur, sulfate and thiol content, and the level of activity and expression of the sulfate transporters and of APS reductase, which all strongly differed in presence and absence of sulfate and/or H_2S as sulfur source for growth, had hardly impact on the development of Cu toxicity in Chinese cabbage.

Conclusions

In Chinese cabbage there was a direct interaction between atmospheric H_2S and pedospheric sulfate nutrition and H_2S exposure resulted in a downregulation of sulfate uptake by the root. There was an upregulation of the sulfate uptake capacity at high Cu^{2+} concentrations (> 10 μM) in both absence and presence of H_2S, though the H_2S-induced partial downregulation of the sulfate uptake capacity occurred at all Cu^{2+} concentrations. Upon sulfate deprivation, foliarly absorbed H_2S could replace sulfate as sulfur source for growth, however, the toxicity of Cu^{2+} was similar to that observed in sulfate-sufficient plants both in absence and presence of H_2S, indicating that the sulfur status of the plant did not have any significance in Cu tolerance. Moreover, the presumed signal transduction pathway involved in the regulation of the expression and activity of the sulfate transporters (APS reductase) appeared to be overruled at high Cu tissue levels.